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"The impulse or desire for a better future":
Comparing Thomas More's *Utopia* and George Orwell's *1984* by John Woo

Abstract

Individualism, privacy, and social norms: these are three important aspects that allow individuals to form a unique identity. In modern times, this discussion of the influence of these themes is becoming even more prevalent as individualism is challenged by the influence from popular culture spread by the internet. Additionally, an individual's privacy is becoming threatened as social media and other apps collect people's data, as well as through the widespread use of surveillance cameras. Social norms are a core part of society due to the great effect it has on one's character and everyday actions. Out of many different types of research mediums, literature is distinctively able to create an emotional impact, allowing readers to walk through a portrayal of individualism, privacy, and social norms. Two novels, 1984 by George Orwell and Utopia by Thomas More, do just that while incorporating these three themes. However, 1984 takes place in a society where these three traits are portrayed in a negative way while Utopia presents them in a positive way to come to a similar effect on readers.

Introduction

Through close readings and literary analysis, this paper addresses the question of how two opposite fictional societies, dystopias and utopias, both cause readers to assess aspects of their current societies and become more aware of what could come in the future if readers are not aware of what is happening in their societies. To answer this question, this research paper will analyze Utopia and 1984 as classics of utopian and dystopian literature. Notably, Thomas More first coined the word "utopia" during the early 16th century, while George Orwell is a prominent political novelist from the 20th century whose book 1984 has been widely read ever since it was first published in 1949. The paper initially analyzes the origins of dystopias and utopias, as genres, with reference to the historical context of these two novels. Then, it explores similar themes across these contrasting genres by presenting an analysis of the lack of individualism, absence of privacy, and contrasting social norms in Utopia and 1984. Through this process, the research will reach a conclusion as to how these three interconnected themes of individualism, privacy, and contrasting social norms, which are present in both novels, are able to produce a

similar impact. Ultimately, the novels, despite their contrasting genres, both lead readers to critique their present society and hope for an improved future.

In many ways, utopian and dystopian novels are polar opposites. After all, a utopian novel portrays an ideal society while a dystopian novel portrays an undesirable one. Yet there is value in comparing books of these different genres. For while dystopian and utopian novels may be seen as opposites, as the title of this essay highlights, "the impulse or desire for a better future is present in each" (Gordin, Tilley, Prakash 2). This paper analyzes two books of these contrasting genres together, *1984* (1949) by George Orwell and *Utopia* (1516) by Thomas More, to show how a desire for an improved society is present in both novels. By comparing these two books, we can see how both *Utopia* and *1984* lead readers to critique their own societies through contrasting descriptions of individualism, privacy, and social norms.

Before going into these three fundamental aspects of modern societies, it is important to elaborate why *Utopia* is being compared to *1984*. *Utopia* is the prominent novel wherein More introduced the term "Utopia": "[he] coined this word from Greek roots, eu-topia (the place where things are well) and u-topia (no place) but it is a word which, overcoming its Latinized-Greek origins, has taken on a life of its own" (Davis 1). The word is now widely used to refer to a "non-existent society described in considerable detail and normally located in time and space that the author intended a contemporaneous reader to view as considerably better than the society in which that reader lived" (Fitting 126). More's novel is about an island society called Utopia, and it introduces unique views of what an ideal society should be through the character Raphael Hythloday's stories of traveling from Europe to the island of Utopia. This positive portrayal is likely to have been influenced by More's historical context because "More's idea of utopia is, in fact, the product of the Renaissance, a period when the ancient world (namely Greece and Rome) was considered the peak of mankind's intellectual achievement, and taken as a model by Europeans" (Vieira 1). This was a time of profound innovation, and the ideal society termed as utopia was influenced by the many successes and optimism of the Renaissance.

By contrast, *1984* presents a dystopia, a detailed, fictional society, whose main objective is to lead the reader to interpret it as "considerably worse than the society in which that reader lived" (Fitting 126). This dystopia is called Oceania, and the main character, Winston Smith, seeks the truth in this totalitarian society full of censorship and brainwashing. Orwell, thus, presents a warning to his readers by portraying the kind of society that may develop if they are

not careful about the figures to whom they choose to give power. While *Utopia* is a founding book for utopian studies, *1984* is studied widely in high schools due to its famous portrayal of a dystopian society and its educational aspect for world history classes as a novel that was written during the period after World War II. Ultimately, through their differences, *1984* and *Utopia* both lead readers to question aspects of their current society.

In the utopian and dystopian societies created by More and Orwell, individualism, the freedom to create a unique and independent identity, is limited for citizens; however, the attitudes toward this are different in each book. By presenting this social trait through different methods, each novel raises questions about the value of individualism. For instance, the Utopians have a set time at which they work, eat, and rest. This daily routine includes: "work three hours before noon when they go to lunch. After lunch they rest for a couple hours, then go to work for another three hours" (More 45). All of their working and resting hours throughout the day are planned out. Yet these strict, structured working hours are portrayed as ideal by Hythloday, who states: "Their working hours are ample to provide not only enough but more than enough of the necessities and even the conveniences of life" (46). Although there is a lack of individualism in this society, Hythloday portrays this as ideal and enjoyed by the Utopians. Thus, it could lead readers to wonder if less individualism would be better for their society.

In the future society of *1984*, however, the lack of individualism is portrayed much more negatively. This contrasting portrayal shows another side of the impact of individualism. Again, working hours and occupations are given to citizens without a choice. It could even initially seem ideal that the Outer Party members are assigned jobs based on their abilities instead of choosing an occupation based on their own unique preferences. However, Winston's job is portrayed negatively. For instance, he sometimes has to work "more than ninety hours in five days. So [does] everyone else in the Ministry" (Orwell 179). Citizens of England during Orwell's time would have had hope for a better future due to the recent conclusion of WWII, but Orwell plays with the readers' expectations by presenting a future characterized by governmentally established long working hours that constrain individuals from having control over their daily routines.

Additionally, the name of the city in which the novel takes place—London, Oceania—shows that Orwell could have been portraying *1984* as a warning specifically for readers in London, England, where the novel was first published. *1984* is set in a period of war

between three superstates, Oceania, Eurasia, and Eastasia. According to the British critic Bernard Crick, Orwell may have incorporated this into the novel because he was writing in "a postwar world [which was] brutally and arbitrarily divided into spheres of influence by the great powers" (Crick 1). With the end of WWII and Nazi Germany, many readers would have been hoping for a future full of individual freedoms and rights. Thus, *1984*'s warning against a divided future with a lack of individualism could have caused readers to be more aware of restrictive elements in their society that could lead toward this dystopian environment.

Apart from individualism, the second aspect diminished in each of these societies is privacy, which would again have likely surprised readers since both novels were written in times of optimism. Privacy is a paramount aspect of contemporary society because it can contribute to the comfort and safety of an individual. Again, More and Orwell take contrasting approaches to this subject: the former presents a positive portrayal of the lack of privacy and the latter presents a negative portrayal. For instance, in *Utopia* the doors of the houses "open easily and swing shut automatically, letting anyone enter who wants to" (More 42). Although the way these houses are designed does not lead to a lack of security in Utopia, readers may believe that they would not be comfortable in this society because of how different it is from what they are used to. The sharing of property contributes to a dramatic lack of privacy, as Utopia has "no hiding places," and there are "no spots for secret meetings" (More 53). Due to customs like these, the Utopians "are bound to be either working at their usual trades or enjoying their leisure in a respectable way" (More 53). The lack of privacy thus leads citizens to act responsibly, which helps society to become more productive and successful. In sum, More portrays the lack of privacy in *Utopia* as a positive aspect of society, unlike Orwell.

In contrast to this positive portrayal, the descriptions in *1984* of the complex paths that Winston and Julia have to take when meeting each other and the impact that the telescreens have on their personal lives convey the constraints of the intense lack of privacy in their society. Winston explains how trapped he feels by the extreme difficulty of meeting Julia, saying: "It was like trying to make a move at chess when you were already mated. Whichever way you turned, the telescreen faced you" (Orwell 110). The simile used here helps to explain how improbable it is that Winston would be able to safely meet Julia due to the surveillance from the telescreen. Similarly, in his apartment, "[a]ny sound that Winston made, above the level of a very low whisper, would be picked up by [the telescreen]; moreover, so long as he remained within the

field of vision which the metal plaque commanded, he could be seen as well as heard" (3). The complex paths and controlling nature of the telescreens highlight the confinement and restrictiveness of Winston's life without privacy.

Moreover, in *1984* this leads to a synthesis between private and public sphere in so far as "[t]he family had become in effect an extension of the Thought Police" (Orwell 133). The children are so loyal to the Party that they would even turn their parents in. This surprising shift in the children's loyalty from their family to the Party creates fear and concern because the children are beginning to do the work of the Thought Police in the home. Usually, the private sphere is a part of life that lacks interventions from the government or an outside force. Yet here private and public are forming together, since the Thought Police are in charge of punishing people who have thoughts that are against the Party, and the children have become an extension of the Thought Police. This is an unexpected relationship because the word "family" usually symbolizes safety and comfort, while the Thought Police can be characterized as invasive and threatening extensions of the government which patrol private thoughts. Whereas the positive portrayal of the lack of privacy in *Utopia* could have caused More's readers to question if that is a more ideal way to run a society, this negative portrayal in *1984* would have inspired Orwell's contemporary readers to pay closer attention to their society so that synthesis of the private and public spheres of society does not become a reality.

Besides the lack of individualism and privacy, it is vital to analyze the contrasting social norms present in both novels because the norms of a society contribute to the ideologies and actions of individuals in a society. "Social norms" are what is socially accepted in a community, and they greatly influence a person's behavior or beliefs. Since social norms can dictate a person's identity and individuality, they are important aspects of a society. In *Utopia*, when a citizen goes to the marketplace, he "looks for what he or his family needs, and carries off what he wants without any sort of payment or compensation" (More 50). Since the society of Utopia is structured around the idea of the commonwealth, everything is shared and its citizens hypothetically own everything. The novel explains that these "customs must necessarily result in plenty of life's good things, and since they share everything equally, it follows that no one can ever be reduced to poverty or forced to beg" (More 53). These unexpected social norms eliminate the social value of objects that was prevalent in England during More's time and values

items based on their utility. When describing this commonwealth, Hythloday makes it sound very ideal.

For instance, precious objects like silver and gold have no value in Utopia. Since the Utopians do not think of these metals as valuable, "their chamber pots and all their humblest vessels… are made of gold and silver. The chains and heavy fetters of slaves are also made of these metals" (More 55). Consequently, when a group of ambassadors from another society visited Utopia, "they were decked out in all the articles which in Utopia are used to punish slaves… [and] all the onlookers considered this pomp and splendor a mark of disgrace" (57). Here, More pokes fun at people who prize materialistic items like jewelry since the people in Utopia prioritize practical items instead. This change in the value of items relates to the historical context since, in the 16th century, England was slowly transitioning into a more commercial economy with the influence of trade and manufacturing. More wrote about these practices in *Utopia* not to convince England to immediately integrate them, but to highlight how "those Utopian practices which were fantastic consistently indicated a practical line of conduct" (Ames 179). By removing the social norms that attribute value to products that most readers would have seen as valuable and prized, More encourages readers to question whether a society that does not value these items would be better.

In *1984*, the norms that the Party establishes also greatly contrast with what many readers might take for granted as good or natural social norms, like the act of "doublethink," which citizens of the society are required to perform whenever a previously accepted fact is altered. This makes a big impact on society because the act takes away the individual's rights to form their own opinions and unique outlook. "Doublethink" is an act that must be done by all Party members and it means "[t]o know and not to know, to be conscious of complete truthfulness while telling carefully constructed lies, to hold simultaneously two opinions which cancelled out, knowing them to be contradictory and believing in both of them, to use logic against logic…" (Orwell 35). When the Party changes a historical fact, although the citizens knew what the truth was at one point, they are expected by the Party to remove that memory from their minds and instead to believe what the Party tells them in the present. The Party incorporates this expectation into society, demolishing the individual's ability to believe in the real truth and to have personal opinions. The alteration of truth in this novel could have been influenced by what happened to the historical record of Leon Trotsky. For instance, "[t]he Soviet Encyclopaedia… first had

Trotsky as a hero of the Civil War, then condemned him as an agent of the Mensheviks and British Intelligence, then dealt with him in the simplest and sweetest way by removing him entirely from historical record, making him an un-person" (Crick 7). Likewise, in *1984* the Ministry of Truth is in charge of changing and rewriting history. *1984* presents a society that requires the lack of real truths and unique opinions due to strict rules established by the Party, leading readers to be cautious about social norms that may inadvertently echo or lead to the unappealing practices of doublethink.

With undesirable practices like doublethink, *1984* takes place in a dystopian society that shares three key themes with *Utopia*, even though the latter uses an ideal society to portray these themes much more positively. Despite the contrast in method and genre, both novels have strongly impacted their readers. While opposite genres in their approach, when "considering utopia and dystopia as linked phenomena, we are able to consider just how ideas, desires, constraints, and effects interact simultaneously" (Gordin, Tilley, Prakash 2). This paper explores certain linked phenomena across these genres by presenting descriptions of the lack of individualism, absence of privacy, and contrasting social norms in *Utopia* and *1984*. These are three important aspects of a modern society that allow individuals to form a unique identity. They are also interconnected with each other as social norms and privacy both influence an individual's identity. Through a negative portrayal of these aspects, *1984* impacts its readers by creating a warning against what may happen in the future if people are not careful in managing their own society. By contrast, due to its positive portrayal of the three aspects, *Utopia* leads its readers to question if the way of life presented in this novel is better.

By analyzing these utopian and dystopian novels together, this paper has shown how two contrasting methods are able to lead to a similar impact: readers becoming more aware of what is going on in their own societies. Apart from the novels' impacts during the time when they were published, each still has a lasting impact on today's readers. *1984* has an enduring impact because it portrays aspects of society that remain relevant, like the widespread use of surveillance cameras. Additionally, the way social media and other apps collect people's data, actions, and information also, mirrors the role of the telescreen in the dystopian society. Readers today can learn from both *1984* and *Utopia* by using the novels to deliberate over what can be done to improve our own societies. Ultimately, both novels are able to present nuanced, unexpected societies through their literary techniques and key elements.

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Pakistani Youth in the U.S. Taking a closer look at a minority group in America by: Iba Baig

Abstract

The Pakistani community is one of a number of minority communities in the United States of America. This community consists of both those who are immigrants and those who are born and raised in America. Being a minority community means that there is a lack of research and understanding of the community, which is further hindered by stereotypes. In order to comprehend this community, it is important to observe the youth who identify as Pakistani American. In this paper, Maryam and Kiran, two female youths in South Miami, Florida, share their perceptions of their identity. They maintain certain aspects of Pakistani culture, such as speaking Urdu, wearing traditional clothing, eating Pakistani food, and doing charity work for the community. Moreover, Islam is frequently a part of many Pakistani traditions. Maryam's faith in Islam allows her to wear a hijab. In contrast, Kiran is connected with Islam through poetry. This paper shows how the characteristics from their Pakistani identity are integrated with their American identity. They wear American clothing on a regular basis, listen to American music, and have more American friends than Pakistani ones. Being a Pakistani-American consists of the struggle to balance both aspects of their identity, but creates a beautiful and unique experience filled with rich culture and background.

Introduction

The minority group of Pakistani-Muslim Americans in the United States and their culture is widely unknown due to their small population. For this reason, it is important to better understand their perceptions on the integration of their culture in American society. Specifically, this paper seeks to understand how Pakistani female youth in South Miami, Florida perceive and understand their social and cultural identity. As a minority group in a diverse city, there is a challenge to one's identity and the way in which it is influenced by their surroundings. By investigating the perspectives of Pakistani American youth, their social and cultural identity is uncovered and allows for a better understanding of the youth in this community. The interviewees discussed how others perceived them, how they perceive themselves, and how they confronted obstacles related to their identity as Pakistani and/or Muslim. Also, Islam influences Pakistani cultural identity in different ways, depending on the individual. Additionally, Pakistani

American youth find ways to develop their identity through actively participating in the Pakistani American community, by interacting with friends in and outside the community, engaging in fashion, indulging in food, listening to music, and even writing poetry.

Methodology

Ethnography was used to collect data for the purpose of this study. Ethnography is the study of cultures to understand a particular subject . Through ethnographic research, the collection of information from participants of the Pakistani-Muslim female youth community in South Miami enables a better understanding of the lifestyle and practices of the community. The study consists of two informal interviews, a collection of observations, and participatory action. Two participants were selected to be interviewed[1] in order to learn about their perspectives of their social and cultural identity. These participants were selected because they are acquaintances. It was easier to contact them since they have my contact information as well. Furthermore, it was difficult to find new people online who were willing to answer personal questions. Also, the participants are people who I met in person and became friends with, so it was easier and more comfortable to discuss this topic where they opened up about their identity. This resulted in more thorough and less filtered answers to the questions. In addition to the interviews, I incorporated observations from a food drive at Masjid-Al-Noor, a renowned mosque in South Miami, to provide some insight on the activities and life of the Pakistani community (Pakistani teenagers included). It is also important to understand the surrounding of a place where many people gather, particularly in the event of weddings, funerals, Ramadan[2], and Eid[3]. However, going to these types of events was not possible because of COVID-19 which limited the opportunities to go out to big gatherings.

The interviewees selected were Maryam, age 17, and Kiran, age 19. Both of them were born and grew up in South Miami, Florida where they are part of and engage with the Pakistani and/or Muslim community. The interviewees both have knowledge of the culture and religion, being more or less active in each sector. Throughout the interviews, Kiran and Maryam were asked a series of questions regarding their personal, family, religious, and cultural life. They

[1] For interview questions, refer to the Appendix.
[2] Ramadan is a month from the lunar calendar that Muslims follow where people fast everyday from sunrise to sunset. It ends with a celebration of Eid.
[3] There are two types of Eids that Muslims celebrate: Eid-Al-Adha (celebrating Hajj and pilgrimage) and Eid-Al-Fitr (celebrating the end of Ramadan).

were given a choice to elaborate on whichever question they wanted and could skip questions they felt uncomfortable with. Both of them were asked to sign a consent form before the interviews were conducted.[4]

For the interviews, the method used was phone-calling Maryam and face-to-face calling Kiran. The face-to-face call enabled me to observe Kiran's facial expressions while answering the questions, which allows for the tone to be interpreted. For instance, emotions were displayed briefly when the interviewee responded. On the other hand, there is a loss of a second scene of communication through the lack of facial expression observations through the phone-call method. The limitations of a phone-call restricted the full aspects of an interview. Furthermore, it was important to explicitly state things in order to get the intended message across. When this occurred in the interview, I asked additional questions for clarification. This was to ensure that no information was being hindered and that all of the answers would be more detailed.

Background

In the United States of America, the Pakistani population is in the minority. There are high concentrations of Pakistanis in metropolitan areas, such as New York, Houston, Washington, and Chicago. As of 2019, there were a total of 554,000 Pakistani Americans in the entire U.S, either immigrants or native born.[5] The youth (ages 5-17) make up 19% and young adults (ages 18-29) make up 21% of the total population of Pakistanis, this includes foreign and native born.[6]

[4] For consent form, refer to the Appendix.
[5] Pakistanis in the US Fact Sheet, Pew News. April 29, 2021.
[6] See graph "Among Pakistanis in U.S." from Pakistanis in the US Fact Sheet, Pew News.

	All Asians in the U.S.	Among Pakistanis in the U.S.		
		All	U.S. born	Foreign born
MEDIAN AGE (in years)	34	31	14	41
AGE				
Younger than 5	7	7	19	1
5-17	17	19	43	6
18-29	18	21	26	18
30-39	17	17	8	21
40-49	14	13	3	19
50-64	16	16	1	23
65+	11	8	1	11

Figure 1: This data table represents the percentage of Pakistanis based on age groups (Pakistanis in the US Fact Sheet, Pew News).

A significant contribution to Pakistani culture and life is religion. The major religion in Pakistan is Islam, where 96.2% of people are Muslims, according to the 2017 census.[7] Furthermore, in the United States, Muslims make up 1.1%, an estimated 3.45 million people, of the total population. Islam is one the fastest growing religions in the world and, by 2040, it is approximated that Muslims will replace Jews as the nation's second-largest religious group after Christians. By 2050, the Muslim population in the U.S. might reach 2.1%, nearly double of the current amount of Muslims.[8] The integration of religion in Pakistani life is a major source of influence on culture, lifestyle, and norms.

Since there is no official data released on the Pakistani population in Miami, Florida, it can be reasonably inferred that they are a minority group. In fact, data regarding the Pakistani population in the U.S. in general is lacking. This may be because of the fact that they are a minority group and no certain research has been conducted to quantify the Pakistani population. With the absence of information on this specific community, I relied on my own data collection

[7] "Religious minorities in Pakistan," Taylor & Francis Online.
[8] "New Estimates show U.S. Muslim population continues to grow," Pew Research, January 3, 2018.

and personal observations. Based on this, I found that in South Florida, from Fort Lauderdale to Miami, the Pakistani population is spread out in different neighborhoods such as Weston to Miramar, and to Kendall. I also concluded that the majority of Pakistanis live in neighborhoods in North Miami. However, the two Pakistani youth I interviewed live in South Miami.

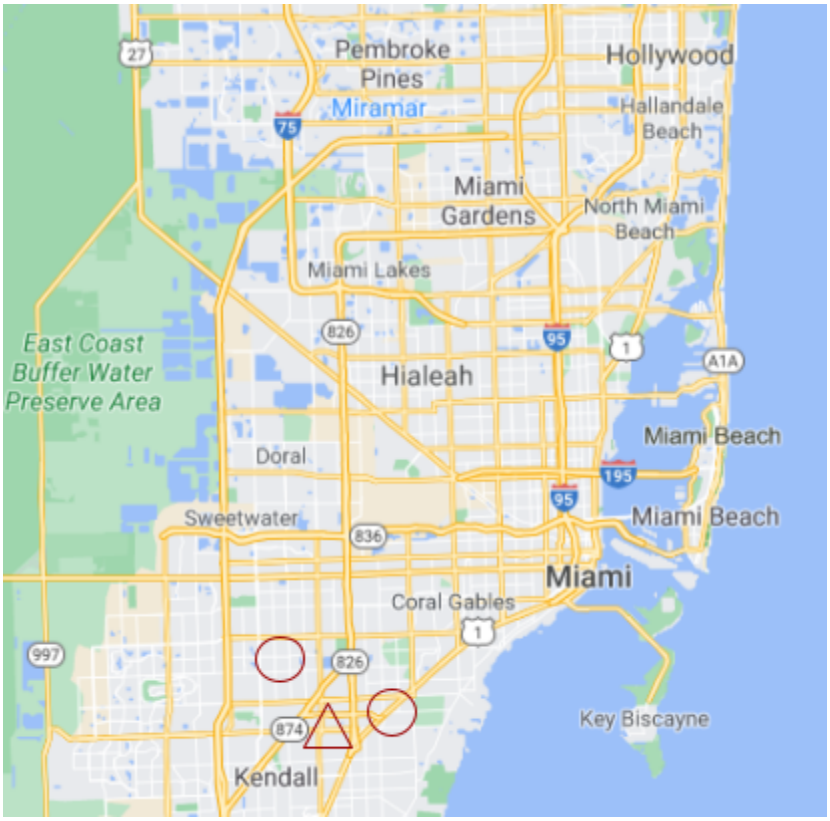

Figure 2: Map of Hollywood to Miami, Florida (Google Maps, May 21, 2021). The circles mark where the participants live. The triangle marks where I live.

Findings

Through the interviews, Maryam and Kiran's perspectives about their cultural and social identity was discussed. Maryam has graduated high school and is going to go to a college in Miami to study the field of medicine. She was born and raised in Miami, Florida, in a Pakistani immigrant family. Kiran is also born in Miami, Florida and both of her parents are immigrants from Pakistan. She now attends the University of Yale and is studying science. The two have some commonalities, one being that they are bilingual within their household.

Language

Maryam, for instance, mentions how she speaks with her parents in Urdu, her native language, whereas with her sister, she speaks English. Kiran mentions that her parents speak to her in Urdu, but she replies in English and that she speaks English with her siblings. Kiran also mentions that her parents want her to be fluent in Urdu, in order to be more aware of her culture, so she finds herself practicing occasionally with them. Both of them feel that it is more natural to speak to their siblings in English than in Urdu, since it is commonly what they speak. Maryam and Kiran frequently alternate between two languages in their household, which is also known as code switching. Code switching is the practice of alternating between two or more languages and reflects how it is seamless for bilingual people to change languages in a conversation within their household when speaking to their parents and their siblings.

Music

In addition to speaking Urdu at home, both participants occasionally listen to Pakistani music. Maryam only listens to Pakistani or Bollywood music at gatherings. She listens to Pakistani singers like Rahat Fatih Ali Khan and Atif Aslam. Also, she knows the lyrics to all of the classic songs from growing up with that music, however, she does not listen to it on her own time, and opts for songs in English, since she can understand them better. This is because she is mainly surrounded by friends who speak English and it is the common language outside of her household. On the other hand, Kiran listens to desi[9] music on her own time and has her own playlist of songs that she likes. One of her favorite songs is called "Suraj Hua Maddham" by Sonu Nigam, Alka Yagnikm, and Sandesh Shandilya. For Kiran, music is another aid in connecting with her culture.

Religion

Another topic discussed with the participants is religion. This was not a commonality between the two participants. Their perceptions and practices of religion were quite different. For example, Kiran said she is not religious and has visited a mosque once, but not for religious ceremonies. She also doesn't pray or read the Quran, but she does fast during Ramadan if she is

[9] Desi refers to people of Indian, Pakistani, or Bangladeshi descent.

able to. In another interesting part of the interview, Kiran mentioned her poetry is the closest she gets to being involved in the Muslim and Pakistani community. She frequently writes and publishes poetry about various self-reflective topics, including Islam. Writing about Islam was sparked by her growing interest in spirituality and religion. Not to mention, her interest in it was driven by her father's past, who was raised in a religious environment while he was growing up but does not practice Islam anymore. Kiran's interest in the religious stories her father used to tell her is reflected in her poetry. For example, a poem she wrote about Islam is called, "The Closest I Will Get to Mecca," and was published in the Yale literary magazine (see poem sample below). This idea that Kiran mentioned in her interview reflects how religion and culture means different things to different people, and is reflected in the unique ways each individual person practices it.

The Closest I Will Get to Mecca.[10]

is a photograph of my father
in my hands.

His ghost of a smile,
quiet, severe. His skin,
wrapped in white.

When I was twelve, I was scared to say
Prophet Muhammed
out loud. Shame

has always howled
into
the hollow of me.

Tonight, my fingers are dusk-

[10] Masroor, Kiran. "The Closest I Will Get to Mecca." May 24, 2021. www.instagram.com/poemsbykiran

heavy on your
temple.

Who named a bone a temple?
Who could be that kind?

Dear God, sometimes I swear I see you
in the sky, the way fish see us

in the abstractions of light
dancing above them.

Dear God, last night, I heard a fist of wind
knocking at my window, asking to be let in.

Was that
you?

Dear God, my dad visited you once,
and then came back. I think

if he tried to see you again,
you won't let him go so easily.

On the other hand, Maryam and her family are religious and frequently attended *Masjid-Al-Noor* pre-pandemic. Most notably, Maryam started wearing hijab, a head-scarf, like her older sister and mother currently wear. She mentioned that she wore the *hijab* to the grocery store once, to see how she felt, and realized that she felt normal. Then, she finally committed to wearing the *hijab* full time, for her spirituality and dedication to Islam. The stereotype that everyone in the Pakistani community is religious, is not necessarily true. Both Maryam and

Kiran have different perspectives of religion, and practice it to various degrees. Therefore, it may not be a factor in every Pakistani youth's social and cultural identity.

Figure 3: Masjid Al-Noor and Islamic School of Miami (Photographer: Iba Baig, January 2, 2021).

Traditional Clothing

One important factor in the participants' social and cultural identity is wearing traditional clothing. All of the participants found that wearing traditional clothing made them feel uncomfortable, especially when people outside of the community, who were uneducated of their culture, were disrespectful about it. Fortunately, they have many other friends who are supportive and interested in the clothing. Kiran mentions that she loves Pakistani clothing because it is different, although she feels more comfortable and confident in western clothing because she is commonly surrounded by it. Furthermore, she perceives it to be a large part of the beauty

standard that she follows. Kiran also mentioned that she feels more "Pakistani" when wearing the clothing, indicating that she feels closer to her culture when she is dressed in traditional clothing. Maryam and Kiran both do not wear Pakistani clothing that often. They only wear it when they go to special occasions, such as a wedding, or casual gatherings where everyone who attends is Pakistani. In the interview with Maryam, she brought up the significance of wearing traditional clothing. She said, "...When I wear traditional clothing, I feel like a real Pakistani."[11] This reinforces the notion that Pakistani clothing is one of the factors that brings teenage youth closer to their culture. It can be inferred by Maryam and Kiran's insight that some Pakistani female youth feel closer to their cultural and social identity not just by practicing in religious or community gatherings, but also by dressing in traditional clothing.

Figure 4: Traditional Pakistani clothing at a wedding. (Photographer: Iba Baig, January 2, 2021).

Charity Work

Another commonality between the participants is how they participate in the community by doing charity work and volunteering to help the community. For instance, Maryam volunteers at the Islamic School of Miami every week (pre-COVID). She also contributes a few dollars

[11] Maryam, Interview, Phone-call, Florida, January 31, 2021.

every time her parents are giving zakat[12], to fulfill her roles as a Muslim and a Pakistani. Kiran said that she is a part of "Yalies for Pakistan,"[13] a club at Yale University. Her role in this club is to create fundraising opportunities. A recent event was a virtual concert featuring Pakistani singers to raise money for COVID-19 relief. She mentioned that as for the Muslim community, she does not do anything in particular for it.

Cultural Foods

Last but not least, Maryam and Kiran are avid lovers of Pakistani foods. In both of their households, they typically eat traditional Pakistani dishes such as biryani[14], curry, daal[15], butter chicken gravy, and naan.[16] Maryam's favorite dish is beef biryani and Kiran's favorite dishes are butter chicken gravy and chicken biryani. They usually eat Pakistani food when they are at home. Maryam says that her family always eats Pakistani food because it is flavorful and her parents know how to cook Pakistani food well. Kiran's parents also are better at cooking Pakistani dishes, since it is what her parents grew up with. Both of them said that they will always continue to eat Pakistani foods and will one day learn to cook the traditional dishes by themselves. Pakistani cuisine is another aspect that keeps them connected with their culture.

Summary

Maryam and Kiran share some similarities, but they also have many differences in the aspects of their culture. Both of them are bilingual in their household, they both love traditional Pakistani cuisine, and they share a similar perspective on traditional clothing. Also, they actively participate in the community through volunteer charity work. On the other hand, in the religious aspect of Pakistani culture, they have different experiences. Maryam practices Islam and wears a *hijab*, while Kiran reflects on Islam through her poetry.

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[12] Zakat is a religious duty for all Muslims to contribute part of their wealth to help the poor.

[13] A student organization at Yale University that celebrates Pakistanis and holds fundraisers or charity drives to help the Pakistani student community and the outside community. Yalies for pakistan. @yaliesforpakistan https://www.instagram.com/yaliesforpakistan/?hl=en.

[14] A rice dish with various spices that can include vegetables or meat.

[15] Lentils.

[16] Oven baked flat bread typically eaten with gravy.

Limitations

Instead of relying on in-person data collection, the ability to use digital ethnography was helpful. With the quarantine restrictions put into place because of the COVID-19 pandemic, the research was strictly limited to digital ethnography. The interviews were conducted over the phone and face-to-face calling. Not to mention, only two interviewees were able to participate in the questionnaire process, as it was difficult to coordinate with other possible participants who were hesitant to talk about their social and cultural identity. In addition to the digital interviews, there were scarce options to visit gathering places of Pakistanis in South Miami, including mosques, such as Masjid-An-Noor, and the local Pakistani grocery store. In order to learn more about the community, the owner of the grocery store was reached out to, but due to a language barrier was unable to go through with. On the other hand, the mosque is open to visitors with proper social distance and masks, so I was able to take pictures and observe a food drive that took place at the mosque.

Although the struggles of digital ethnography limited some data collection, there are also many advantages of digital data collection. First and foremost, it is convenient to conduct interviews over-the-phone, without the struggle of having to commute to a location. Also, there are also a lot of online databases that provide access to an abundance of information. However, with the topic of the study being the Pakistani community in Miami, there is a scarce amount of research in this field. This may be due to the fact that they are a minority community and that there might not have been a need to collect information from this particular community. Although, this situation allowed me to center my findings around the interviews and the observations.

Conclusion

There is no certain standard of the perceptions Pakistani youth have about their cultural and social identity. Each and every person expresses their identity in different ways, whether it is through participating in the community (including family, friends, and charity work), wearing traditional clothing, indulging in food, practicing religion, listening to music, and even writing poetry. The contribution of the insight from the participants, Maryam and Kiran, expands the scholarship that both breaks and reinforces some stereotypes or understandings about the involvement of Pakistani people in their communities.

One example is that being a Muslim does not necessarily mean you worship at the mosque frequently in order to feel connected to Islam. For example, Kiran does not attend the mosque while Maryam does. In addition, Maryam wears a *hijab* and prays five times a day whereas Kiran explores Islam through her poetry. Another stereotype that is broken is that being a Pakistani American does not necessarily mean you feel more comfortable in traditional clothing. Maryam and Kiran have a shared view on traditional clothing, which is that they feel more comfortable and confident in Western clothing, but they also love wearing Pakistani clothing on occasion.

Both seem to share a similar experience: they adhere to their Pakistani traditions while integrating it with their American identity. The coexistence of being Pakistani and being American creates a unique identity that a minority of youth in America share.[17] Although, there is a struggle between being "too American" from the perspective of a Pakistani or being "too Pakistani" from the perspective of an American. In other words, the individuals in this community try to find a balance between their cultural and social identities, as Maryam and Kiran were able to share.

[17] See Figure 1: graph of "Among Pakistanis in U.S." from Pakistanis in the US Fact Sheet, Pew News.

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Appendix A.

Interview Questions:

a. Name?
b. Age?
c. School & Hobbies:
    i. Can you tell me about your interests?
    ii. What ethnicity are your friends?
        1. Do you have Pakistani friends?
        2. Can you tell me about your Pakistani friends?
d. Family:
    i. Did your parents immigrate to the states or were they born here?
    ii. Where are they from?
    iii. Do you visit Pakistan often?
        1. If yes, how often? Can you tell me about your time there?
        2. If not, why?
    iv. What languages do you speak at home: to parents, to siblings?
        1. Why do you speak different languages in the same household?
e. Religion:
    i. Do you visit the mosque often?
        1. If yes, Can you tell me more about it?
            a. Who do you go with? What do you do when you are there?
            b. Islamic school?
        2. If no, why not?
    ii. Do you pray?
        1. If so, how often do you pray?
        2. *If not, how come?
    iii. What are your thoughts on zakat?
        1. What do you do to help out your community?
        2. Have you helped out before at the mosque?
f. Traditions:

i. Clothing: What are your thoughts on traditional clothing?
    1. What reactions do you get?
    2. Do you want to wear it?
        a. Do your parents make you wear it?
    3. How often do you wear it?
        a. Where do you wear it?
    4. How do you feel when you wear it?

g. Community:
    i. What do you do to participate in the community?
    ii. How do you feel about the community?

Appendix B.

Sample Consent Form

Study Title: Pakistani Youth in Miami
Researcher: Iba Baig
Version Date: 1/15/2021

Participation is voluntary
It is your choice whether or not to participate in this research. If you choose to participate, you may change your mind and leave the study at any time. Refusal to participate or stopping your participation will involve no penalty or loss of benefits to which you are otherwise entitled.

What is the purpose of this research?
The purpose of this research is to study the population of Pakistani youth through the usage of ethnography in order to better understand the community.

How long will I take part in this research?
Your participation will involve one interview with the possibility of 1-2 more follow-ups.

What can I expect if I take part in this research?
As a participant, you will take part in interviews and be present at places where I conduct my observations.

What are the risks and possible discomforts?
If you choose to participate, I foresee there not being any risks or discomforts.

Are there any benefits from being in this research study?
We do not expect any direct benefits to you from your taking part in this research.

Will I be compensated for participating in this research?
You will not be compensated for participating in this research.

If I take part in this research, how will my privacy be protected? What happens to the information you collect?

If you agree, the interview will be audio recorded. The content of the recording may be transcribed. Part of this content may be quoted in the paper I will write. Your identity will remain confidential. Only my Polygence mentor and I will know your identity. Before we begin the interview, please let me know if you agree to do this interview. This statement will also be recorded to ensure that your agreement has been obtained. The recording will be deleted at the end of the project, and I will not use your real name in my paper. I will retain the paper in my personal files indefinitely.

The information with your name on it will be analyzed by the researcher and may be reviewed by a Polygence mentor checking to see that the research is done properly.

If I have any questions, concerns or complaints about this research study, who can I talk to? The researcher for this study is Iba Baig who can be reached at: _____. The Polygence mentor is Dr. Lulie El-Ashry who can be reached at _____if you have questions, concerns, or complaints:

If you would like to talk to the research mentor,
If you think the research has harmed you,
If you wish to withdraw from the study,
If your questions, concerns, or complaints are not being answered by the research team,
If you cannot reach the research team,
If you want to talk to someone besides the research team, or
If you have questions about your rights as a research participant.

I have read the information in this consent form. All my questions about the research have been answered to my satisfaction.

SIGNATURE

Your signature below indicates your permission to take part in this research. You will be provided with a copy of this consent form.

_____

Printed name of participant

_____

_____

Signature of participant Date

Research and Current Theories of Neurodevelopmental Disorders Through Organoids

by Tanisha Joshi

Abstract

Neurodevelopmental disorders affect brain development and function, resulting in a wide range of variability in symptoms, such as ASD, and are thus challenging to treat. One such example is autism spectrum disorder (ASD), for which there is currently no known cure. Additionally, diagnosis of ASD is difficult, as the disorder manifests as a spectrum of behavioral symptoms. Current diagnosis options include EEG scans and behavioral reaction tests, such as to determine neurological conditions and activity. Neurobiologists have studied the etiologies and treatments (or therapies) of ASD through animal models, such as mice. However, animal studies are limited in their applications to humans, leading to the development of new technology to study human-specific biology. Organoids are artificial organs that can be grown from cultured stem cells and used as a model to study disorders/diseases in neurobiology, such as ASD. In this article, I will discuss the history and current research on neurodevelopmental disorders such as autism and the history of organoids. In doing so, I will review how dysfunction in the nervous system can lead to altered plasticity, the theorized mechanisms of neurodevelopmental disorders such as ASD, and the advantage of organoids in addressing unresolved questions about such disorders.

Introduction

Through several years of research on neurodevelopmental diseases, neurobiologists and psychiatrists have educated millions about the background of ASD along with delivering current and new treatments to patients. Advances in technology have enabled new techniques to model systems, such as organoids, which have the potential to better understand brain mechanisms underlying disease and disorders. As there is currently no medical treatment for ASD, it is important to properly model the disorder to understand how ASD functions and impacts patients in a mental and physical way. Based on the history of the brain and its functions, along with the history and current research of organoids, this paper aims to determine if using cerebral organoids would be fit as a way to model neurodevelopmental disorders, specifically ASD.

In this article, I will provide context on the normal functions of the brain, including concepts such as plasticity and receptor function along with how each impacts neural circuitry through a review of past experimental research. I will also describe the history of diagnosing and treating ASD and discuss current theories of how and why ASD occurs while reviewing how the development of organoids and the significance of their contribution to neurobiology research thus far. Finally, I will address how organoids can be leveraged to model ASD.

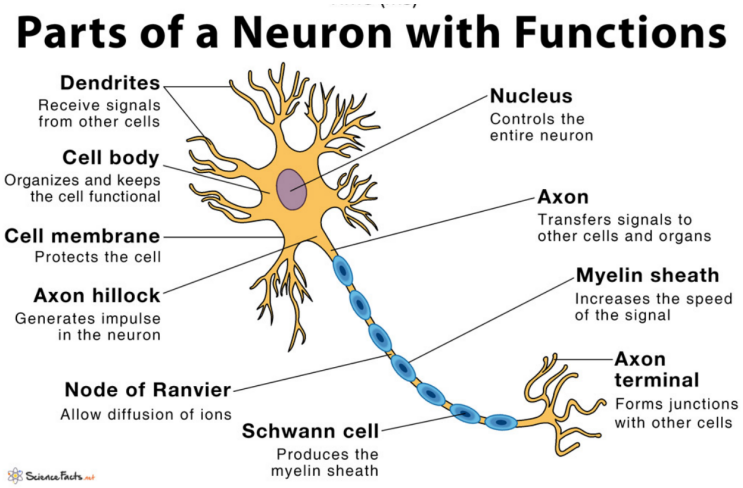

Figure 1: Parts of a neuron with functions

Background

In the healthy brain, electrical stimulation is required in order for a *neuron*, or nerve cell, to fire. The dendrites of a neuron are responsible for receiving information in the form of electrical stimulation, the cell body is responsible for processing this signal, the axon helps transport the processed information across a neuron, and the axon terminals send the information to the next cell **(Fig. 1).** This phenomenon is called an *action potential.* The electrical nature of an action potential is important because it enables rapid neural communication in the nervous system. The firing of action potentials in specific patterns allows organisms to process information and execute behaviors **(Fig. 2).** The place where two neurons meet to transfer the signal is called a *synapse*. There are two types of synapses, chemical and electrical.. In chemical synapses, a specialized cell connects two neurons to help transfer information, which is done so through the release of neurotransmitters from the presynaptic terminal (the neuron giving information.) In electrical synapses, information is transferred through a connection between the

two neurons, called gap junctions. Synaptic plasticity relates to the change in the strength of the synaptic connection between neurons and its ability to change when facing alterations or challenges in the pathways of the brain. Famous neuropsychologist Donald Hebb worked to understand the connection between psychology and neuroscience, resulting in a rule that is widely used in the field today. According to "Hebb's Law", he confirmed that a synaptic connection can continue to strengthen through synchronized firing patterns that are specific to certain synapse types. Receptors play an important part in making sure that the synaptic connections function properly, especially NMDA receptors and AMPA receptors. These receptors regulate excitatory synapses and help neurotransmitters and messengers release signals from a presynaptic neuron, which increases the chances of an action potential in the postsynaptic neuron. Depending on the protein the NMDA receptor binds to, the process of LTD (long-term depression of a neuron) or LTP (long-term potentiation) occurs **(Fig. 3).**This controls whether or not the strength of a synaptic connection is weaker or stronger, respectively.[1]

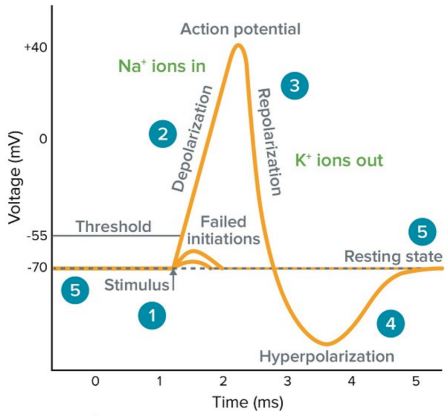

Figure 2: Action Potential Phenomenon

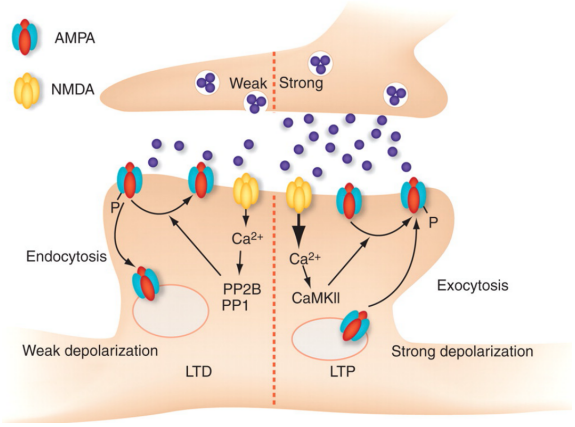

Figure 3: NMDA and AMPA Receptors

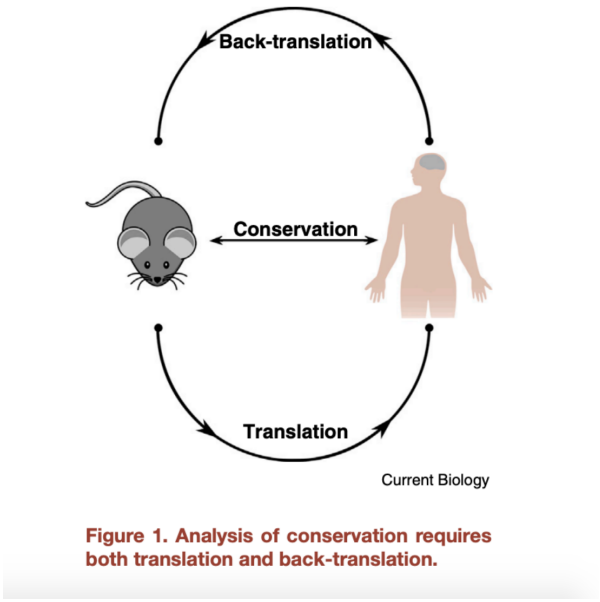

Figure 1. Analysis of conservation requires both translation and back-translation.

Figure 4: Translation and Back-translation Model

Many theories in neuroscience are often tested in mice, which are used as model organisms because they share similar characteristics with humans. For example, mouse and human genomes have similar coded proteins while sharing similar functions in organ regulation. This reflects the concept of translation, which means that if a treatment successfully works in mice, then it has the potential to work in humans because of the similar biological compositions of both organisms. Human-tested drugs can also be effective in mice in a back-translational way because of their genetic similarities, meaning that if medicinal treatments are effective in humans, then they should also be in mice. Neurobiologists argued that because many treatments, regardless of whether they were developed in mice, back-translate to mice, mice are a good model organism **(Fig. 4).** However, through clinical trials, scientists have reported low success rates of translation in experiments and attributed the cause to a lack of knowledge about the mechanisms of neuropsychiatric diseases in mice. Therefore, organoids present a unique advantage: reducing the usage of mice in research along with experimenting with human-derived cultured tissue to model human-specific neurobiological disorders. Furthermore, more applicable research on neurodevelopmental developmental activity and reactivity to various stimulants and stress cues could be investigated and experimented on.[2]

History of Diagnosing and Treating Autism Spectrum Disorder (ASD)

When first understanding and investigating autism, doctors typically diagnosed autism on the basis of behavioral symptoms. Through research and assessments, doctors have correlated repetitive motions and inflexible communication as symptoms of autism. Further investigation by psychiatrists and neurologists resulted in the discovery that patients with the neurodevelopmental disorder had biologically different brains compared to people who did not have autism.[3] These neurobiological changes are thought to alter the development of the human brain and underlie ASD behaviors. Autism was discovered to be a heterogeneous disorder, meaning there are several causes that lead to having autism. While there is a major genetic predisposition in patients who have been diagnosed, ASDis also influenced by environmental risks, such as birth trauma or hypoxia. Various tests help confirm diagnosing autism in patients; the two main ones being behavioral and technological tests. In technological tests, EEG (electroencephalography) tests are primarily used to determine the level of brain function in people with autism. Importantly, these tests can also show how synaptic changes occur in the brain due to a genetic condition.[3] Another machine used is an MRI machine, which helps doctors see how the brain is functionally and structurally different due to disrupted pathways in the neural circuitry.[3] Both of these machines help diagnose autism by evaluating the brain's electric activity. Behavioral diagnosis procedures entail doctors to evaluate the child's developmental history to get a deeper understanding of the specific interactions the child has with peers and adults. Through the EDSM (Early Start Denver Model) model, autism management is observed and practiced. Another procedure is the Picture Exchange Communication System which aids in speech generation with training in behavior-related tasks. Drugs and medicine aren't normally used to specifically cure autism, but few are used to manage out-of-control or severe behavioral problems. Typically, behavior-related treatments have been applied as they are deemed to be the safer and most approachable route, rather than experimenting with drugs and other types of medicine.

Current Theories on the Neurobiological Basis of ASD

While investigating different occurrences of neurodevelopmental diseases in the brain of a patient, it is necessary to take the neurobiological history into account, along with their interactions with other vital organs in the body. This provides a deeper understanding of the

relation between brain functions and neural disorders such as ASD. In a properly working neural system, excitation and inhibition (E/I) are signaling processes that occur in cell firing. As mentioned earlier, excitatory currents encourage action potentials to fire to continue sending information while inhibitory currents do the opposite. Having a proper E/I balance in the brain is necessary for the normal function and regulation of bodily processes. In fact, many neurodevelopmental diseases are correlated with sensory or somatosensory dysfunction, in which E/I balances are impaired.[4] Sensory dysfunction is a type of condition that occurs in patients who have ASD or other neurodevelopmental disorders, affecting the way the brain reacts and processes sensory stimuli and information. Patients who have SPD (sensory processing dysfunction) are often overly sensitive to objects and sounds that they encounter in their nearby environment, impacting how they react to one or multiple senses.[4] Somatosensory dysfunctions are similar to sensory dysfunctions in that the impairment affects the way the brain processes sensory information but on a more touch and pressure basis. For example, patients who have somatosensory dysfunctions would be sensitive to different measures of touch, movement, vibrations, etc. Dysfunctions like these correlate to brain developmental issues but could also occur in patients who have had a stroke or other injuries that physically damaged their brain in some way.[4]

Gut-Brain Axis and Somatosensation

Somatosensation can be affected by natural biological processes in the human body through different nerves and pathways that are interconnected. One example is the gut-microbiota axis, which plays an important role in the brain and behavior. The human microbiota is made up of microorganisms that aid in developing and regulating systems in the body, such as the nervous, immune, and metabolic pathways. The gut microbiota brain axis is the main pathway utilized by the human body to help the gut communicate with the central nervous system (CNS) in a bidirectional method through indirect and direct signaling with lipid proteins.[5] Another way that the gut-microbiota communicates with the CNS is through the vagus nerve, which facilitates the transmission of information to different organs in the body from the brain, specifically connecting the brain and the stomach. Disruptions in the gut microbiota through bacteria or other cells have been known to be a factor that causes neurodevelopmental disorders, including ASD. If the system cannot properly regulate homeostasis in the gut microbiota axis,

major problems could occur in the CNS which eventually leads to failure of the proper development of the brain. Individuals with ASD or other neurodevelopmental diseases are known to have gastrointestinal dysfunction as well, suggesting that the gut microbiota axis and the vagus nerve are necessary factors to help maintain the proper functioning of the brain and body. When it comes to minimizing the effects of somatosensory dysfunctions, there is currently a lack of effective methods that are capable of doing so.[5]

Genetics

Specific genes, such as Shank3 and Mecp2, are correlated to ASD as many neurodevelopmental diseases are often gene-related. The loss of function of those specific genes often signals the development of ASD. An experiment was conducted in mutant mice that modeled ASD, in which they were exposed to the chronic treatment Shank3 and Mecp2 as a way to improve their behaviors and social impairments. As a result, the scientists did in fact see improvements in the levels of their anxiety along with amelioration of abnormalities in their brains as well. Using the process of translation, this treatment could dampen some of the effects of sensory dysfunctions in humans. Currently in the field, ASD is thought to involve E/I imbalance. In order to correlate ASD and the importance of E/I balance, an experiment was conducted in vivo to test how excitation and inhibition affect the synaptic plasticity of the brain along with seeing how it plays a role in the overall brain circuit of a mouse with ASD.[6] Contrary to the findings before, the experiments found that changes in excitation and inhibition may actually be required to homeostatically stabilize firing rates, thus the change in E/I ratios may actually compensate for other underlying neural circuit dysfunction.[7]

Development and Leveraging of Brain Organoids

Scientists have used a variety of animal models in their experiments. For example, action potential and its generation were originally discovered through squids. Similarly, the frog was used to study synaptic transmission because of its easily measurable synapses **(Fig. 5).** However, in general, mice are the most commonly used animals in experiments because of their biological similarity to humans as they are both mammals. As stated previously, mice are used as translational models for testing medication in humans.[8] However, as technology continues to develop, there are more ethical and advanced options to model research regarding neuroscience

and neurodevelopmental processes or diseases. Organoids, or replicas of organs that are grown via cultured stem cells, are revolutionized as being the next way to display and model different diseases.[9] Stem cells is a term for a special type of human cells that can turn into different cell types. Organoids are typically grown in a lab where the environment can be adjusted, usually in incubators in laboratories.[9] The usage of organoids may help further revolutionize the way diseases are modeled in the future.[10]

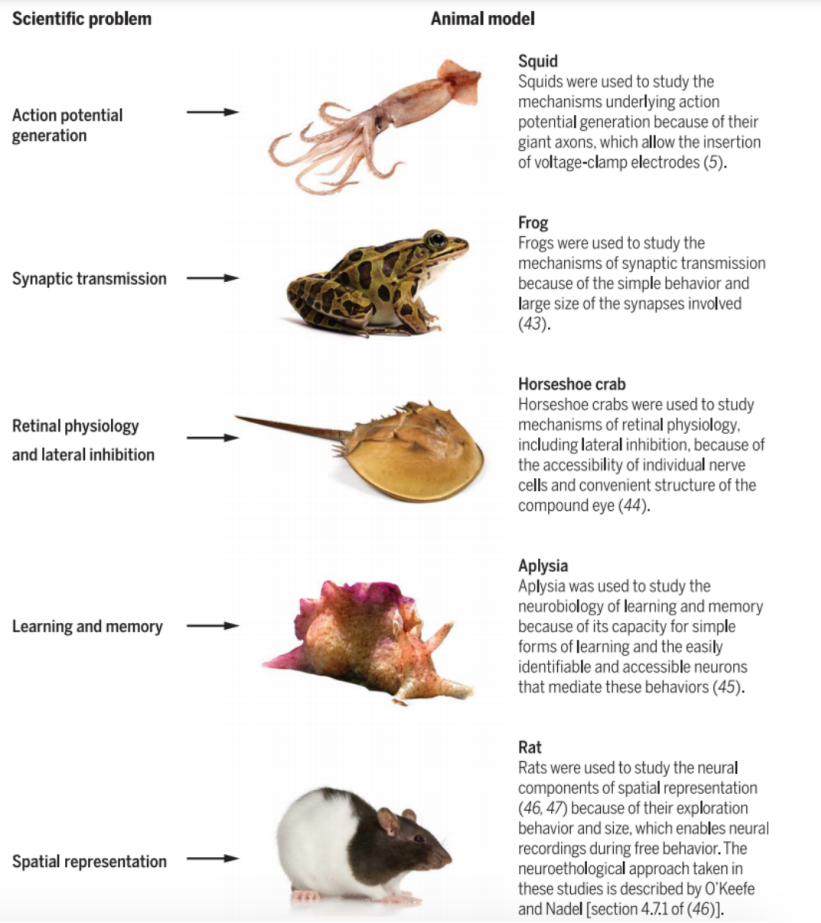

Figure 5: Animal Research Model

Through the utilization of organoids, various neurodevelopmental disorders can be modeled through the stem cells of the diseased tissue and be a way to model other polygenic or monogenic diseases. Polygenic and monogenic refer to the number of genes that are playing a part in the causation of a disorder. For example, multiple genes that cause the disease would be polygenic while a singular gene that causes a disease would be a monogenic disease. However, the use of organoids can support the development of treatments for neurodevelopmental and vision problems **(Fig. 6).** Organoid technology has massively grown in the past few years,

leading to in vitro studies of disorders in brains that are in embryos.[11] While culturing stem cells in one form of organoid development, another method by the name of *CRISPR*, a method of genetic alteration, is also a tool used in the reconstruction of organoids.

Reconstructing organs through organoids requires a vast amount of technology and different branches of science as well, including transcriptomics, proteomics, and epigenomics. *Transcriptomics* is the study of genomes and the RNA transcripts it produces while *proteomics* is the study of proteins that are used by cells and organisms. E*pigenomics* is the study of correlating genetics and the environment. All of these branches have an important role in understanding how organoids function and how they would manage to replicate actual organs using derived stem cell tissue to grow and nurture. Spatial transcriptomics methods identify cell types and find out how specific biological occurrences and processes occur in that specific region of the tissue.[12] Organoids enable scientists to potentially explore specific regions in the brain and conduct experiments regarding synaptic connectivity, E/I balances, or determine the overall structure and function of the brain as a research mechanism to find prospective solutions for neurodevelopmental disorders.

CRISPR, a method of genetic alteration, is also a tool used in the reconstruction of organoids, along with finding out changes in the composition of cells in pathways. CRISPR allows editing and alterations of genome sequences in humans which could be used to correct genes incorporated in diseases or model those specific diseases through physical organoids to see the regions that are impacted in a physical sense. *Perturbed cells* (genetically altered cells) are often used in CRISPR to identify genes that are necessary for the specific diseases that are being studied. *iPSCs* (induced pluripotent stem cells) are cells taken from humans that are converted to iPSCs, which can then be grown into organoids in the lab. These cells can be genetically modified via CRISPR to test experimental questions in the developed organoids. Some scientists have been successful in experimenting with CRISPR in ASD and autism-related testing. In this experiment, scientists wanted to figure out how ASD risk genes affect cellular processes through an in-vivo CRISPR method. By placing a frameshift mutation in altered cells that were displaying ASD in mice, they identified that there were 14 gene modules that expressed various cortical cells, meaning that the mutation altered the cells in the brain and affected cellular processes. This further helped scientists to understand that organoids and CRISPR can be utilized to model ASD or do a deeper analysis to get a better understanding.[13]

Takeaway

As psychiatrists and doctors have researched autism over the past few decades, many great discoveries have been made that helped patients and the public learn more about the history and causes of autism. However, despite the achievements, there is still much to be learned about autism in terms of establishing a permanent solution and utilizing technology while doing so. As technology continues to evolve and researchers continue to broaden their horizons in the neurobiology field, more research will be conducted that will continue to evolve and help millions of people in the future. The overall takeaway from this research is that it is important to properly model neurodevelopmental disorders through the usage of organoids to understand how ASD functions and impacts patients through various stages in the diagnosis procedures while creating probable solutions while doing so.

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Drug Discovery Using Machine Learning and Data Analysis by Aida Fakhry

Abstract

Estrogen receptor-positive (ER+) breast cancer is the most common subtype of breast cancer worldwide [1]. This research paper targets the different methods questioning the binding to ERα promoting breast cancer proliferation. Primarily, the CHEMBL database was run through the default coronavirus drug discovery and then moved towards the discovery of expanding keywords into aromatase. Centers have approved therapies by aromatase or by modulating ERα. However, besides their therapeutic success, they induce several side effects and unknowns (regarding dosages).

Aim

The function of this research will focus on finding a compound (or a molecule) that will be able to inhibit the function of the aromatase enzyme. For the focus of this research the organisms that will be tested through the following data structure will be the aromatase enzyme [4]. Considering that aromatase activity and ERα display significant roles in breast cancer, the goal is to discover compounds able to simultaneously inhibit aromatase and modulate ERα [part of the cytochrome p450, which is responsible for breast cancer].

Methods

I used the ChEMBL Database through Python to retrieve all the known aromatase inhibitors (AIs) and ERα antagonists. Using the ChemAxon software, I computed two types of compounded organisms. Selected compounds were thoroughly analyzed by molecular docking. Anti-aromatase [stopping the production of estrogen in postmenopausal women] activity in the Database was evaluated in human placental microsomes and ERα expression was assessed [3].

Under the CHEMBL database, there are 2 single proteins under the “target_type” and the rest are Organsms [figure 1]. This was found after changing the target search to “coronavirus” and changing target_query = target.search to input (‘coronavirus’) instead of the default. This omitted difficulty for precise drug discovery.

[Figure 1]: Rendement of Target ID

	cross_references	organism	pref_name	score	species_group_flag	target_chembl_id	target_components	target_type	tax_id
0	[]	Coronavirus	Coronavirus	17.0	False	CHEMBL613732	[]	ORGANISM	11119
1	[]	SARS coronavirus	SARS coronavirus	14.0	False	CHEMBL612575	[]	ORGANISM	227859
2	[]	Feline coronavirus	Feline coronavirus	14.0	False	CHEMBL612744	[]	ORGANISM	12663
3	[]	Human coronavirus 229E	Human coronavirus 229E	12.0	False	CHEMBL613837	[]	ORGANISM	11137
4	[{'xref_id': 'P0C6U8', 'xref_name': None, 'xre...	SARS coronavirus	SARS coronavirus 3C-like proteinase	9.0	False	CHEMBL3927	[{'accession': 'P0C6U8', 'component_descriptio...	SINGLE PROTEIN	227859
5	[]	Middle East respiratory syndrome-related coron...	Middle East respiratory syndrome-related coron...	9.0	False	CHEMBL4296578	[]	ORGANISM	1335626
6	[{'xref_id': 'P0C6X7', 'xref_name': None, 'xre...	SARS coronavirus	Replicase polyprotein 1ab	4.0	False	CHEMBL5118	[{'accession': 'P0C6X7', 'component_descriptio...	SINGLE PROTEIN	227859

In this research project I will be examining the bioactivity for SARS coronavirus 3C-like proteinase (index number: (4)th entry on [Figure 1]). Beneath this, there will be a Target ID: ‘CHEMBL3927’; note that this is the unique identification of the target.

[Figure 2]: Filter Set For Accurate Drug Discovery

```
activity = new_client.activity
```

```
res = activity.filter(target_chembl_id=selected_target).filter(standard_type="IC50")
```

The second filter following the “res” variable should set the standard_type to IC50 [Figure 2]

The second filter following the “res” variable should set the standard_type to IC50 [Figure 2]. Thus, when filtering out the selection within the CHEMBL database, IC50 will be chosen for the column type. IC50 will be used for this research as it is the most widely used and informative measure of a drug's efficacy. IC50 (half-maximal inhibitory concentration) indicates how much drug is needed to inhibit a biological process by half. This provides a measure of potency of an antagonist drug in pharmacological research [2]. By running this under df.head (or, due to the lengthiness of columns, you can set () to (3)), you can access “standard_type” and select “IC50.” To access the unique values in this column, type in “df.standard_type.unique().” Notably, the only “unique” type for the dataset is IC50.

Before further examination of the “standard_value,” it is essential to understand the notion of potency with each drug. I.e., on the first column the potency for IC50 is 7200.0. The lower the number, the better the potency of the drug, vice versa. The ideal finding within drug discovery in the CHEMBL database is a high potency. In other words, the inhibitory

concentration would be at 50%, thus having a low concentration. The ideal finding will ensure that there will be a drug that produces the same inhibition at 50% as a different drug but at a lower dosage. Notably, the bioactivity data is in the IC50 unit. Compounds having less than 1000 nM will be considered to be active while those greater than 10,000 nM will be considered to be inactive. As for those values between 1,000 and 10,000 nM will be referred to as intermediate.

Each compound will be described by a molecule CHEMBL ID (molecule_chembl_id) - each row represents one compound. Though, there may be instances in which multiple rows will contain the same molecule chamber ID. For simplicity and undeviating results, I will keep only one of them. To best ensure there is no redundancy, iterate/run a for loop under "canonical_smile," "molecule_chembl_id," and "standard_value."

[Figure 3] Target Search For Aromatase:

```
target = new_client.target

target_query = target.search('aromatase')

targets = pd.DataFrame.from_dict(target_query)
targets
```

Switching target_query = target.search to target_query = target.search('aromatase') [Figure 3] switches the keyword to an enzyme as part of the cytochrome p450, which is responsible for breast cancer.

[Figure 4] Result From Target Search:

	Cross_references	Organism	Pref_name	Score	Species_group_files	Target_chembl_id	Target_components	Target_type	Tax_id
0	[{'xref_id':	Homo	Cytoch	19.0	False	CHEM	[{'acce	SINGL	9606

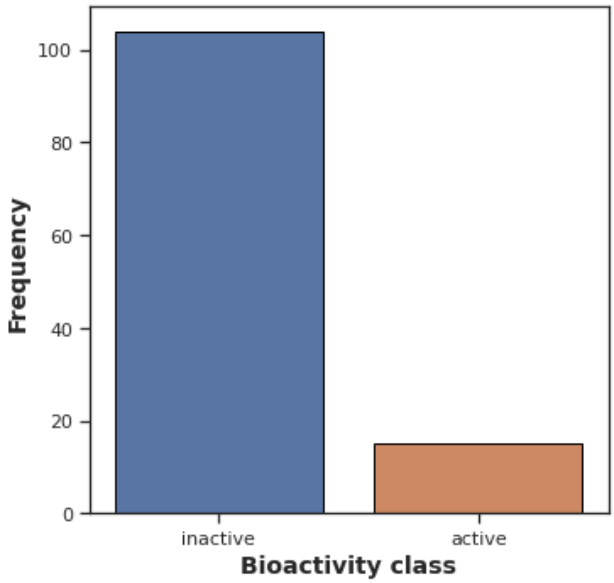
	'P11511', 'xref_name': None, 'xre...	sapiens	rome P450 19A1			BL1978	ssion': 'P11511', 'component_description...	E PROTEIN	
1	[{'xref_id': 'P22443', 'xref_name': None, 'xre...	Rattus norvegicus	Cytochrome P450 19A1	19.0	False	CHEMBL3859	[{'accession': 'P22443', 'component_description...	SINGLE PROTEIN	10116

By running ‘aromatase’ within the variable target_query, the outcome for pref_name is Cytochrome P450 19A1. When running ‘rattus norvegicus’, the outcome for pref_name is also Cytochrome P450 19A1. Notably, the species group files are both false, and the target type are both singular. Thus, a fair prediction before analyzing data is that the potency of both organisms may be fairly close in results. Additionally, the p-value may be quite low - or even ‘null.’

Computing Molecular Descriptors [Testing Hypothesis]

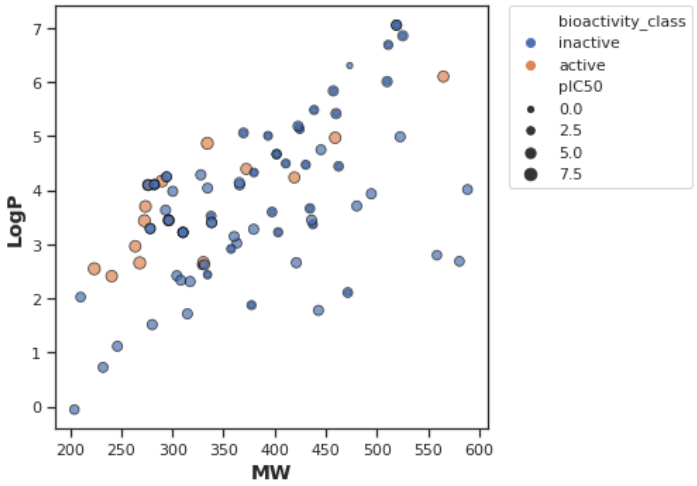
Running the code cell for installing conda and rdkit are essential during drug discovery because they allow you to compute the molecular descriptors for the compounds that have been compiled through the download (the data set of biological activity from the CHEMBL database). This computation will be measured using molecules and smiles notation (which is the information about the chemical structure), in order to compute the molecular descriptors. IC50 will be used once again as a bioactivity class: active, inactive, and intermediate. During this drug discovery active and inactive compounds will be monitored. The lipinski (drug likeness of a

compound) which is based on the Absorption, Distribution, Metabolism, and Excretion (ADME) will be used to see whether the drug can be a.) absorbed by the body or b.) distributed in the body, and become metabolized.



[Figure 5]: Seaborn Importation

This figure is made from importing “matplotlib.pyplot as plt” under the seaborn importation. Both bioactivity classes are shown to compare the inactive and the active molecules within the drug.



[Figure 6: Scatter plot of MW versus LogP]

It can be seen that the 2 bioactivity classes are spanning similar chemical spaces as evident by the scatter plot of MW vs LogP. The distribution of the inactive and active class is expected because of the threshold of 5 and 6. In other words, if the pxc 50 > 6, it will be active, if pxc < 50, it will be inactive. Inactive: 1-5: Active: 6-7.

Mann-Whitney U Test [mannwhitney('pIC50')]:

The Mann-Whitney U Test was run to determine whether there is a statistical significance for the pIC50. As a result, the p-value is rather low (1.662636e-10) therefore, the hypothesis that the p-value would be "null" is rejected. Interpretive results based on running all four chemical space's via Lipinski descriptors: only LogP exhibited no difference between the active and the inactive, whilst the other three descriptors comprising of: MW, NumH-Donors, and NumHAcceptors.

Combining X and Y variable [CSV File Creation]

After downloading the CSV file (that contains combining x and y variables to the dataset), the model building begins. The target protein for this model building is acetylcholinesterase (a cholinergic enzyme). For simplicity, bioactivity data 3 (class pxc50) essentially tells us that it contains the bioactivity data information, comprising three categorical classes: active, inactive, and intermediate. For measurement, PubChem FP to determine the fingerprint - this becomes useful when simultaneous fingerprint data is used.

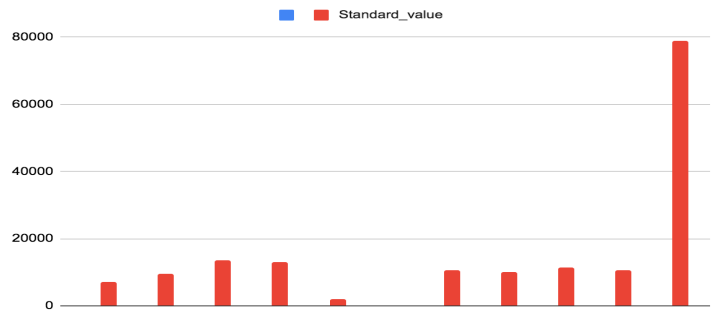
Input Features

Unique "fingerprints" within the database represent unique molecules. The unique properties of the compounds should be distinguished between compounds that are inactive and active. This will answer whether or not some drugs are potent (good) or not good (target variable: pIC50). Upon dropping (x = df.drop('pIC50, axis=1)), the results will be focused on pubchem fingerprints [Figure 7]. Furthermore, the tenth and fifteenth ranked features (i.e. PubChemFP804 and PubChemFP741) correspond to 3-sulfonyl phenol which according to the SMILES from its substructure description, seems to fit as a part of 4,4′-sulfonyldiphenol (Bisphenol S) (National Center for Biotechnology Information, 2020).

Building a Regression Model using Random Forest

model = RandomForestRegressor(n_estimators=100)
model.fit(X_train, Y_train)
r2 = model.score(X_test, Y_test)

Output Data [Conclusive Data Chart]:



Conclusion

Conclusively, the data supplies information that proves the theory that the dosages within ERα are enhanced to a level that can be reduced, further researched, and broken down into a lower dosage. Trends between active and inactive compounds were found through descriptive analysis which qualitatively and quantitatively addressed issues by further researching the CHEMBL database and QSAR model. ER activity prediction was evaluated via machine learning algorithms and several functions (classes) of fingerprint descriptors. This research paper proves that aromaticity, amine groups, and aliphatic hydrocarbons are important for the active compounds. Moreover, ERβ should be examined thoroughly in relation to ERα. The knowledge gained from this study serves as general guidelines for data-driven machine learning drug discoveries.

Acknowledgments

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To What Extent Did Political Pressure Influence Film Portrayals of Communism in the United States During the Cold War?" by Simran Sharma

Criterion A: Identification and Evaluation of Sources

This investigation will explore the question, “To what extent did political pressure influence film portrayals of communism in the United States during the Cold War?” The first source being evaluated is "The Film Industry's Battle against Left-Wing Influences, from the Russian Revolution to the Blacklist," as it was published in the premier journal for research on film history, acknowledged for its academically thorough articles. The second source, *Showbiz Politics: Hollywood in American Political Life*, has been widely regarded as uniquely insightful regarding the manipulation of entertainment as an American political tool for nearly a century.

The first source is a journal article written by Larry Ceplair for *Film History* in 2008. The origin of this source is valuable because Ceplair is an American author and former professor of history. Having written a plethora of works focused on the motion-picture blacklist and anti-communism, his specialization in the field and background in history establishes his credibility in this investigation’s subject matter. However, the origin of the source is limited because Ceplair grew up in LA. His proximal immersion in Hollywood may have skewed him to favor the film industry, a very progressive field, prompting political bias towards communism.

The purpose is to detail to the audience the progression of anti-communism in the political culture of America through the first and second Red Scares, as it related to Hollywood. This is valuable because it directly analyzes the translation of political pressures on the film industry into depictions of communism. Nonetheless, the purpose has limitations, as it recounts events from the first Red Scare, which started in 1919, whereas this investigation centers around the Cold War, which started in 1947, encompassing a scope of work several decades beyond the focus of this investigation. The content of the source is valuable because it provides information about government actions and events in the film industry, detailing the progression of political sentiments in films at the time.

The second source is a book written by Kathryn Cramer Brownell. The origin of this source is valuable because the book was published relatively recently, in 2014. Brownell’s temporal distance from the historical events described in the book allows for the text to benefit from the objectivity of hindsight. However, the origin of the source is limited because as a

secondary source, Brownell's interpretations of the motives behind historical figures are largely conjectures. Since it is not a primary account, explanations of thought processes are surmised, rather than definite: for example, the reason for celebrity involvement in politics.

The purpose of Brownell's book is to explore how personal relationships, institutions, and government policies from 1928-1980 established entertainment as a central feature of American politics. This is valuable because it provides insight into the connections between Hollywood and politics, and the government's use of media as a tool. However, there are limitations in its purpose, as Brownell focused heavily on how Hollywood affected politics, emphasizing celebrity political culture, somewhat neglecting the effect of politics on film itself. The content of the source is valuable because it provides direct quotes from politicians who played a part in Hollywood's anti-communist push.

Criterion B: Investigation

The development of cinema has long been intertwined with evolving culture. The culture in the United States (U.S.) was heavily impacted by politics following World War II (WWII), as indicated by the Cold War; a period of tension between the United States and the Soviet Union. While it is profusely accepted that political sentiments towards communism shifted in the U.S. during this period, the disagreement lies in whether political pressure influenced American film's portrayal of communism or if the industry remained independent of governmental forces. Although films from the Cold War era can be viewed as tainted by political pressure in the United States, overwhelming evidence supports that they were maverick pieces in their interpretation of communism.

The perspective that political pressure was not a significant factor in Hollywood's portrayal of communism is reinforced by the industry's self-motivated anti-communism. Industrial corporations were autonomous, often boasting their independence from the White House's prejudices.[18] It is likely that many of them were not manipulated by the government, but rather happened to genuinely agree with their agenda.[19] This explains why, in 1944, it was individuals from the motion picture industry who launched the Motion Picture Alliance for the Preservation of American Ideals, an anti-communist organization.[20] These industry goliaths were

[18] Pierre Sorlin, "The Cinema: American Weapon for the Cold War," *Film History* 10, no. 3 (1998): 376.
[19] Ibid., 378.
[20] John Sbardellati, *J. Edgar Hoover Goes to the Movies: the FBI and the Origins of Hollywood's Cold War*, 70.

not coerced into following guidelines which buttressed the second Red Scare, but rather sought to create an organization themselves. It is likely that they truly adopted McCarthyist principles, especially as wealthy individuals who wished to promote capitalistic values. Addressing the capitalist nature of cinematic success, anti-communism can be argued as a simple response to markets. Regardless of whether or not the war altered the consciences of movie producers, their films continued to be, as they had always been, the result of safe and profitable formulas.[21] Anti-communism was an easy sell in Cold War America, reflecting the culture of the people, rather than political pressures. The portrayals of communism were likely reflective of apathy in many cases; they were merely a marketable response to a transformed audience.

Further bolstering the argument that political pressures were not significant in Cold War era depictions of communism in U.S. films, there was an abundance of continued subversion and resistance to government ideology. The film *The Russians Are Coming, the Russians are Coming* (1966) embodies the rejection of compliance in the film industry at the time. Both painting Russians in a positive light and satirizing America's Cold War hysterics, the production was applauded with both commercial success and critical acclaim.[22] The existence and success of this film proves that examples of subversive portrayals of communism were not being politically curtailed. This lack of significant U.S. political pressure is further epitomized by the filmmakers' open communication with the Soviet Union during the film's writing process.[23] Not only did they portray communism positively, but they were free to incorporate the authentic Soviet perspective.

Resistance to government ideology in film was also present in response to government intervention. When proof of CIA interference in Hollywood filmmaking was brought to light in the mid-1960s, a "storm of protest" erupted in the US.[24] This backlash reflected a desire for authentic film. The government's dependence on private media-entertainment for Cold War propaganda was their own downfall - the government often did not have the right to actually enforce policies that would put political pressure on companies; as such, they would exercise their freedom.

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[21] Larry Ceplair and Steven Englund, *The Inquisition in Hollywood: Politics and the Film Community, 1930-1960* (Berkeley: University of California Press, 1983), 312.
[22] Tony Shaw, "The Russians Are Coming The Russians Are Coming (1966): Reconsidering Hollywood's Cold War "Turn" of the 1960s." *Film History* 22, no. 2 (2010): 242-243.
[23] Ibid., 247.
[24] James Chisem, "U.S. Propaganda and the Cultural Cold War," E-International Relations, last modified August 20, 2012, https://www.e-ir.info/2012/08/16/u-s-propaganda-and-the-cultural-cold-war/.

Conversely, it can be contended that political pressure influenced film through direct governmental involvement in the industry at the time. The Cold War was characterized by the emergence of the second Red Scare, a phenomenon that enhanced anti-communism in the United States, situating the motion-picture industry as a medium for anti-communist propagation.[25] Giving way to the 1947 coalition between the Federal Bureau of Investigation (FBI) and House Un-American Activities Committee (HUAC), the director at the time, J. Edgar Hoover, is noted stating, "I have always felt that the greatest contribution this Committee could make is the public disclosure of the forces that menace America—Communist and Fascist."[26] Their investigation of "Communist Infiltration of Hollywood Motion Picture Industry" began on May 12, 1947, in Los Angeles, and eventually led to the hearings of a group of writers and directors known as the Hollywood Ten, in October 1947.[27] All ten were convicted and blacklisted from Hollywood, exhibiting a blatant attempt by the government to eliminate any dissidents who would portray communism in a way which strayed from their agenda. Aside from just legal involvement, the Central Intelligence Agency (CIA) interfered in the production of Hollywood films in the early Cold War.[28] The jurisdiction the organization held over these films allowed them to peddle a politically charged anti-communist message.

Furthermore, political pressure manifested itself beyond direct action, as government sentiment. In 1946, the newly-appointed president of the Motion Picture Association of America (MPAA), Eric Johnston, said, "We'll have no more Grapes of Wrath, we'll have no more Tobacco Roads, we'll have no more films that deal with the seamy side of American life. We'll have no more films that treat the banker as villain."[29] As the head of the MPAA, he had the dominion to effectively serve as a government mouthpiece, imposing the ideals of the regime which he had just served under, as the president of the U.S. Chamber of Commerce. Furthermore, the government rewarded public figures such as Jack Warner, Walter Wanger, Ronald Reagan, and George Murphy, for asserting their status to advocate anti-communism.[30] These activists engaged in political activity to dispel concerns of entertainment's sullying power,

[25] Larry Ceplair, "The Film Industry's Battle against Left-Wing Influences, from the Russian Revolution to the Blacklist," *Film History* 20, no. 4 (2008): 402.
[26] Larry Ceplair and Steven Englund, *The Inquisition in Hollywood: Politics and the Film Community, 1930-1960*, 123.
[27] Kathryn Cramer Brownell, *Showbiz Politics: Hollywood in American Political Life* (Chapel Hill: University of North Carolina Press, 2014), 108.
[28] Chisem, "U.S. Propaganda and the Cultural Cold War."
[29] Sbardellati, *J. Edgar Hoover Goes to the Movies: the FBI and the Origins of Hollywood's Cold War*, 126.
[30] Brownell, *Showbiz Politics: Hollywood in American Political Life*, 104.

ultimately being recompensed with political ties which opened doors to greater authority.[31] Thus, the pressure from the government's increasing scrutiny of perceived political depravity in film pushed individuals within the industry to subscribe to their McCarthyist doctrine.

The aforementioned political pressures were blatantly evident through the increased depictions of anti-communism in films released during the Cold War, as "prior to the second Red scare, anti-radicalism and anti-communism had barely affected movie making in Hollywood."[32] For example, *Red Planet Mars* (1952), a science fiction film, depicts the evils of communism and a fictional overthrow of the Soviet Union, while many other sci-fi films of the time featured world domination-seeking aliens, a glaring parallel for communist infiltration.[33] The 1955 animated adaptation of *Animal Farm*, produced by the CIA, alters the original ending of the book which sees the pigs and humans, metaphors for communists and capitalists, become indistinguishable from each other, which was intended as an allegory for the equal threat of totalitarianism which they posed. Instead, the adaptation leaves only the pigs, or communists, to be critiqued.[34]

The U.S. government vehemently viewed the motion-picture industry as having "vast influence over the lives of American citizens" during the Cold War, deeming it logical that subversives would use the medium to disseminate 'un-American' ideals.[35] However, there is an abundance of evidence which suggests that political pressure did not significantly alter the film industry's portrayal of communism. There were many factors which may have fueled self-motivated anti-communism in the industry, as well as many examples of successful subversive films coupled with vocal rejections of government intervention in the industry. However, there is also ample evidence that political pressure played a major role in the film industry's depiction of communism, with direct intervention exhibiting itself through several government bodies, as well as government sentiments asserting through threatening reinforcement. Ultimately, the presentation of communism in U.S. films during the Cold War can more accurately be attributed to factors independent of political pressure. While cinema in

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[31] Ibid., 9-10.
[32] Ceplair, "The Film Industry's Battle against Left-Wing Influences, from the Russian Revolution to the Blacklist," 403.
[33] Cyndy Hendershot, "Anti-Communism and Ambivalence in "Red Planet Mars, Invasion USA", and "The Beast of Yucca Flats"," *Science Fiction Studies* 28, no. 2 (2001): 249.
[34] Samantha Senn, "All Propaganda is Dangerous, but Some are More Dangerous than Others: George Orwell and the Use of Literature as Propaganda," *Journal of Strategic Security* 8, no. 3 (2015): 152.
[35] Sbardellati, *J. Edgar Hoover Goes to the Movies: the FBI and the Origins of Hollywood's Cold War*, 131.

the Cold War era was highly characterized by Hollywood's engagement in the 'politics of Americanism,' filmmakers acted of their own volition.

Criterion C: Reflection

Through this investigation of the influence of political pressure on Hollywood's film portrayal of communism in the Cold War, I've come to understand the methods and limitations associated with the work of a historian.

In my investigation, I accumulated objective facts from a multitude of sources, then bolstered these specifics with my own analysis, crafting my perspective and reaching a conclusion. Larry Ceplair used this same method in "The Film Industry's Battle against Left-Wing Influences, from the Russian Revolution to the Blacklist." Ceplair analyzed an assortment of facts to properly provide context which he used to frame his narrative.

This method of investigation is, however, limited, as the analysis is susceptible to bias. As a modern-day American, I'm extremely critical about our government's historical wrongdoings and undue interference. Thus, I approached the investigation with a preset disdain for the government. Likewise, Ceplair had to face his propensity to partiality as he is not only American, but also from Hollywood, meaning he is directly immersed in the subject matter.

My observations regarding the methods and limitations used by historians brought forth another question: what constitutes the difference between selection and bias? I found that Tony Shaw, author of "The Russians Are Coming The Russians Are Coming (1966): Reconsidering Hollywood's Cold War 'Turn' of the 1960s," who referenced specific movies which fit his perspective, employed the tactic of selection. By focusing on examples which directly supported the conclusions he presented, he was able to provide analysis which read as unbiased. However, his strategic selection of examples strays from the notion of selection as simply extracting the most important information to provide a focused account. Rather, he cherry-picked to back his claims; still completely based in fact, but not representative of the entire context. In this way, selection and bias are distinct, but can heavily overlap when information is manipulated.

Due to implicit bias, it is impossible for a historian to remain completely impartial; however, it is the role of a historian to separate their biases from their analysis as best they can, to provide an encompassing account which is up to the audience's interpretation. As such, I wholly justified two opposing perspectives with extensive research in my investigation. Despite

my bias towards the film industry, I even ultimately concluded that the U.S. Cold War era film industry's portrayal of communism was not overwhelmingly influenced by political pressure, though this suppresses my personal contempt of what I view as a propagandizing government.

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Leveraging Different Liquid Biopsies Markers For Early Cancer Screening And Monitoring by Phuc (James) Nguyen

Abstract

Liquid biopsy is an emerging method for early cancer diagnosis as it analyzes the patient's liquid in an extremely convenient, minimally invasive and reproducible way. Furthermore, it gives scientists reliable data about the patient's specific cancer, which is pivotal to developing many other individualized therapies. Qualification has been recently granted to circulating tumor DNA (ctDNA) and circulating tumor cells (CTCs) for its use as diagnostic biomarkers for cancer detection. Alternatively, there are many more components or factors that could enhance the diagnostic potential of liquid biopsy, namely tumor-derived extracellular vesicles (tdEVs), circulating tumor-derived proteins, circulating tumor RNA (ctRNA) and tumor-bearing platelets (TEPs). This review will mainly explore both the benefits and limitations of ctDNA and CTC as biomarkers for cancer monitoring. Moreover, I will discuss the current advancements in the process of separating and analyzing circulating tumor biomarkers alongside the usefulness of non-blood liquid biopsies.

Introduction

It is widely proven and agreed that early detection of cancer is fundamental to improving treatment outcomes. Yet, there lacks sufficient techniques for early detection. The current gold standard for tumor detection is tissue biopsy, which comes with many inconveniences such as difficult-to-obtain tissue samples, low accuracy, and expensive costs [1]. Furthermore, it can not differentiate heterogeneous tumors from invasiveness, which makes it overall not suitable for long-term clinical monitoring [1]. On the other hand, liquid biopsy is a detection method that could be easily obtained, cheaper, and non-invasive [2]. Thanks to all of these advantages, it has received numerous applications, from screening early cancer tumors to monitoring cancer progression to analyzing drug response to spotting relapse [3].

Since common liquid biopsy markers (LBMs) serve as cancer biomarkers in the early detection of cancer, it is a fundamental basis and requirement for the proper function of liquid biopsy [4]. The best part is that there are many types of LBMs, which allows scientists to do many tests to increase accuracy [4]. Scientists could analyze ctDNA, CTC, TEPs, and tdEVs

simultaneously to reaffirm the results obtained from one test [4]. Among many developments that enhance the diagnostic and prognostic potential of liquid biopsies, there are still many clinical applications unmet due to certain limitations [5]. Additionally, there remains an inquiry on the possibility of using non-blood bodily fluids as cancer biomarkers [5].

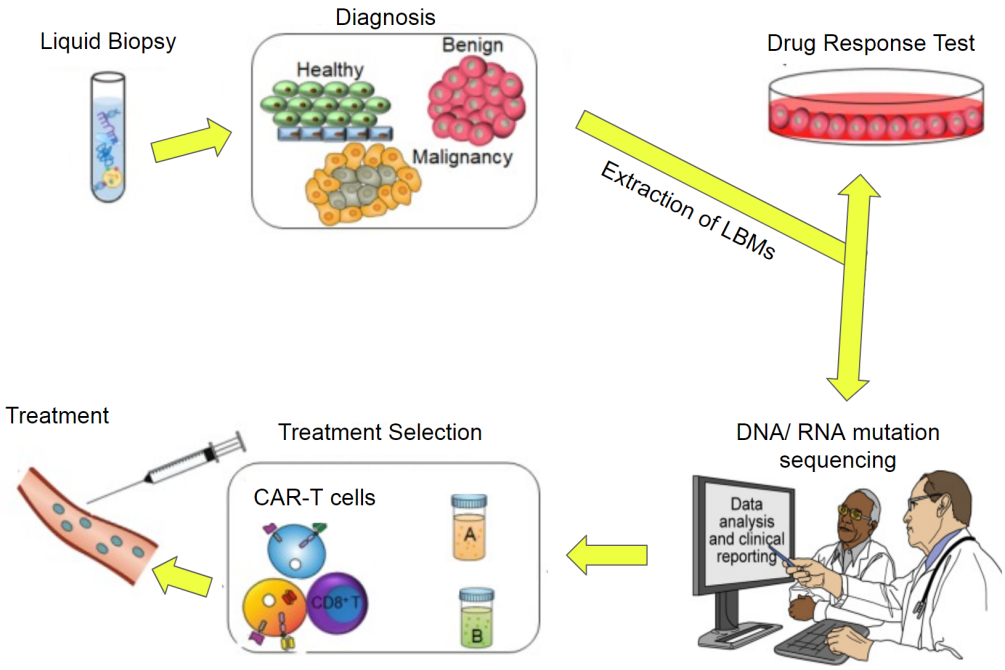

Figure 1: The Clinical Value of LBMs Detection

Circulating Tumor Nucleic Acids

During apoptosis or necrosis, the patient's original tumor produces extracellular nucleic acids which end up circulating in the bloodstream [6]. These tumor-associated ctDNAs/RNAs vastly contrast that of the healthy patient's sera. The mutated KRAS gene, a widely known cancer mutation, actually can be evidenced in the ctDNA captured in the patient's blood [7]. Unsurprisingly, myelodysplastic syndrome and acute myelogenous leukemia (AML) patients' ctDNA also expressed the N-ras mutations [7]. Extracellular-DNA (exDNA) are often indicative of cancer too, especially when they are found in the patient's serum or plasma [8,9]. There are many types of exRNA: microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and messenger RNAs (mRNAs) [9,10]. They can all be very useful playing the role of non-invasive cancer biomarkers [10]. The ctRNA levels detected can help monitor the patient's drug response as it alters regularly following a normal surgery [11]. Detection of gene mutations and

methylation are the two main avenues in which cfDNA/ctDNA technology serves as the major platform. For example, the first clinical ctDNA-detection cancer tests, known as Roche Diagnostics, were able to track the tumor's response to the epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitors (TKIs) [12]. Another approved CRC-using diagnostic test functions primarily on the basis of the SEPT9 gene promoter's pattern of methylation [13]. But, because healthy cells can also contain mutations and genetic variations, the use of liquid biopsy to detect gene mutations alone might not be very useful in terms of early detection. Since almost all patients harbor normal tissue's genetic mutations and only a third of all patients harbor cancer-specific mutations, researchers conclude that the use of the cfDNA/ctDNA's methylation is better than gene mutations [14]. Additionally, when detection of genetic mutations are used for other diseases that have mutations at specific genetic loci such as clonal erythropoiesis, each of the detected mutations need to be sorted and analyzed independently from each other, rendering the process inconvenient and rigorous [15].

Since microRNAs modulate gene expression, they play an important role in many biological/ pathological processes such as tumor initiation and development [16]. Many preclinical studies have found that circulating miRNA are very tumor-specific biomarkers [17]. This means that they can serve as a platform for accurate prognosis and the tracing of the metastasis origin [18]. Different microRNA levels found in the serum describe different physiological conditions [19]. For example, it can be used to detect pregnancy and even the specific stage [20]. Analysis of capture ctDNA mainly uses either PCR or next-generation sequencing (NGS). Meanwhile the first attempt at detecting ctDNA utilizes allele-specific PCR, many modified versions have been developed such as the COBAS® EGFR test [21]. To increase the PCR technologies' ctDNA detection accuracy, many cutting edge versions of PCR have also been developed. Some examples include the ddPCR (droplet digital PCR), BEAMing (beads, emulsion, amplification and magnetics), and dPCR (digital PCR) [22]. These methods are not only highly sensitive, but also convenient and affordable [23]. However, since it is not capable of analyzing bigger gene sequences than a several number of loci simultaneously, these PCR techniques are restrictive in terms of low multiplexing capacity.

The NGS-based ctDNA detection method performs even more poorly since they detect fewer loci compared to other already-restrictive PCR-based technologies [24]. To solve this problem of low sensitivity, researchers use the CAPP-Seq technology, which can spot specific

cancer sequences using a larger available library of cancer sequences [25]. To further enhance the efficiency, researchers attach unique molecular identifiers to distinct template sequences and selective nucleases to healthy DNA, which improves differentiation [26]. Moreover, breakthroughs in A.I involving whole-genome sequencing (WGS) are promising avenues for various forms of liquid biopsies, including ctDNA detection [27]. For instance, the DELFI (DNA evaluation of fragments for early interception) platform is an artificial intelligence-based technology that is able to track cancer progression from early diagnosis to relapse [28]. Firstly, tumor-derived ctDNA are matched with particular previously-known mutations.[28] Next, these ctDNA are compared with the rest of the healthy DNA [28]. From here, researchers measure the fragment length distribution to input all the data into the database [28]. Then, more information such as position of chromosome arms, mutant alleles and AGC abundance were inputted and marked on a scale to serve as a baseline for future improvements [29]. All of this information synergizes to create a specific patient's ctDNA profile, which can be used to conveniently separate the healthy patients from the cancer patients [29]. This DELFI platform performs with a 91% detection accuracy in 236 cancer patients and 98% in 245 healthy patients, proving that artificial intelligence is a promising and powerful tool in the fight against cancer [29]. Despite this, ctDNA detection performs poorly when the patient's cancer is in its earliest stages because the immature tumors produce very few ctDNA. This increase in ctDNA rarity decreases the MAF and detection sensitivity.

Circulating Tumor Cells

During metastasis, the original tumor generates cells to invade nearby tissues or even travel to distant body parts through the bloodstream [30]. These CTCs, often found floating in the patient's blood, create tumor clones and blood vessel walls to support neo-angiogenesis [30]. Since CTCs are indicative of cancer metastasis, it can be used as a reliable biomarker for early detection. From the baseline to the second anti-breast cancer treatment, increased CTCs had been linked to worse cancer prognosis and treatment outcomes [31]. This finding was also confirmed and replicated in other types of metastasis cancer from ovarian to colorectal [32, 33]. Aside from assisting the development of new drugs, the tracking of CTCs population, more importantly, is key to early cancer detection and successful monitoring of the patient's cancer progression and drug responses [34]. Additionally, scientists could use CTCs to separate patients into different

risk groups, matching each group with the fittest (neo) adjuvant therapies [35]. Despite these benefits, CTCs are very hard to spot in the cancer patient's peripheral blood because they are only found at a ratio of usually one per million blood cells. To detect CTC, researchers utilized epithelial markers [36]. Specifically, they use the EpCAM-coated magnetic beads to attract and separate the epithelial cells from the patient's blood. Then, these cells get specified through fluorescently-labeled antibodies [36]. Even though not yet approved, emerging systems, detecting stem-cells-associated CTCs, are extremely useful because they alert the doctors of the ongoing epithelial- mesenchymal transition (EMT) and, sometimes, even myschemal characteristics these CTCs acquired [37]. Traditional CTC detection systems, ones that use epithelial markers, can not detect stem-like CTCs subpopulations, thereby neglecting micrometastasis and metastatic CTCs [38]. So, more research and studies on these CTC subpopulations and how they inform us about their tumor is pivotal to new breakthroughs in therapeutics. CTCs are different in each patient; this allows for more individualized drug screening and treatment administration [39]. Depending on the patient's tumor mutation profiles and drug sensitivity patterns, they will receive the treatments accordingly [39].

Though it gives significant insights for developing clinical treatment, expanding CTCs in vitro is ultimately unuseful due to many reasons. Firstly, there are many factors that could impose unfixable harms to the CTCs' contents, namely enrichment, harsh culture conditions, and pre-sample processing [40]. Secondly, long- term in vitro culture increases the risks of CTCs adopting novel non-tumor-associated traits, rendering the culture a less accurate version of the original tumor [40]. To eliminate this burden, researchers are coming up with ways to upgrade the sorting and enrichment system and the in vitro culture conditions. Furthermore, to understand the mechanisms CTCs use to survive different peripheral blood's components, researchers develop and study the co-culture of CTCs and immune cells or other blood cells [41]. Using these valuable insights, scientists plan to develop new methods to expand the clinical implementation of CTCs beyond liquid biopsy early detection. The most useful aspect of CTCs is their DNA segments that hints back at their original tumor. After CTCs isolation, researchers analyze their genetic components through a variety of techniques including target NGS, dPCR-based mutational spectroscopy technology, genome-wide sequencing technologies, qPCR, and many more [42]. Since CTCs' chromosomal rearrangements are very specific to its tumor, scientists aim to access them by using fluorescence in situ hybridization (FISH), a type of

cytogenetic techniques [43]. To decode how tumor subpopulations interact with its surrounding tissue, researchers use multi omics analyses to dissect the CTCs at the single-cell level [44]. Single CTC genomics and transcriptomics can therefore be considered as an extremely helpful tool in examining tumor heterogeneity. Moreover, it could be used to compare the effectiveness of liquid biopsies versus tissue biopsies.

There are more than one ways of capturing or detecting CTCs: biological and functional. Scientists can apply the strategy that best fits the particular CTC. In the biological method, biomarkers are fundamental. Scientists enhanced EpCAM+ cells and then utilized either cancer biomarkers or CD45+ depletion [45]. In more advanced technology that gives higher CTC yields, the recognition of surface phenotypes are enhanced by microfluidics [45]. To separate CTCs from the patient's normal blood cells, scientists could also look at the difference in size and density [46]. After that, to affirm the initial results, they further examine the CTCs' dielectric properties by a variety of methods, namely di-electrophoresis, microfluidics, filtration, differential centrifugation and many more [47]. In the functional method, assays, which can either employ CAM digestion, capture protein release or analyze the expression of telomerase, are the main platforms [48]. Despite all the advantages that each method has, they still can not sufficiently capture CTCs due to the rare and heterogeneous nature of CTCs. To solve this problem, scientists exploit many methods at once to improve CTC yields.

CTC-Chip

As a technology that receives increasing clinical applications, the CTC-chip takes use of microfluidics in a way that boosts both sensitivity and specificity in separating CTCs from the rest components in the patient's fluid sample [49]. One of its most major strengths is the ability to gently grasp on CTCs, which ensure that isolated CTCs are still in its purest form [50]. As a controlled nano-scale environment that allows for biological-analysis activity that a macro-scale environment would not, microfluidics performs micro-assays, quickly stimulating the fluid to microscopic distances and leveraging the differences in fluid dynamic properties that occurred as a result [50]. These microfluidics enable researchers to manipulate cells and transport reagents [51]. As a chip, made of silicon, with a relatively small size (that of a standard microscope slide), the CTC-chip serves as the basis for bioengineering. On the chip's surface, there are 78,000 μm-sized posts, many of which form geometric patterns [51]. Some of these posts are covered

with the epithelial cell adhesion molecule (EpCAM) to filter the CTCs from the fluid sample [52]. Flow velocity and shear force are two critical factors that influence CTC yields. Faster or slower flow velocity respectively decreases or increases the amount of time that CTC contacts the posts [52]. Similarly, stronger or weaker shear force respectively damages or enhances the maximum cell–micropost attachment [52]. After the patient's blood sample goes towards the surface of the CTC-chip through pneumatic forces, they meet a plethora of microposts [53].

The purposefully-designed geometric arrangement on the chip affects the fluid dynamics so as to ensure that the blood's cellular component travels down a specific pathway, which maximizes the times of contact between CTCs with the EpCAM posts [54]. This way, both the efficiency and effectiveness of the capture process increases. To ensure that the captured cells are CTCs, researchers check the staining which can differentiate leukocytes from epithelial CTCs [55]. After that, CTCs are measured and analyzed at a molecular level. The viability of these CTCs remain unaffected due to the minimal preprocessing required and shear force applied [56,57]. The platform still has many more open avenues for innovations. One promising example is the implementation of different antibodies that could detect different CTCs accordingly, which will lead to a greater availability of studied CTCs, which will overall enhance our understanding of the original tumor [58].

Conclusion

By serving as an affordable, quick, reproducible and non-invasive method of diagnosing and monitoring cancer, liquid biopsies prove to be a useful tool in the fight against cancer. Compared to tissue biopsies, liquid biopsies can examine the tumor's clonal heterogeneity when the patient's circulating tumor-derived factors are analyzed. Scientists can utilize many liquid biopsy samples so as to reaffirm the accuracy of cancer diagnosis. What's more, the following-administered real-time biopsies can do a huge favor by distinguishing the therapy-resistant tumors from the non-resistant ones. Beyond that, liquid biopsy could be used to detect and track the minimal residual disease, usually occurring after initial therapy. Eliminating the need for long and expensive purification processes, automated chip-based devices or CTC chips are upgraded technology that fundamentally applied liquid biopsies to analyze biomarkers in the patient's fluids sample. But, since there is a deficiency in standardized pre-analytical and analytical variables, liquid biopsies are limited to being used at a wide clinical scale. In

summary, liquid biopsies can be a transformative tool, revolutionizing the current reactive cancer treatment paradigm, as it helps oncologists to proactively diagnose, monitor, and develop personalized drugs. Despite all of its potential, there still remains a need for more research and development aiming towards enriching the downstream analysis of circulating biomarkers.

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